

In Vitro-In Vivo Assessment of Oral Absorption of Hydrocarbon Residues

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Outline

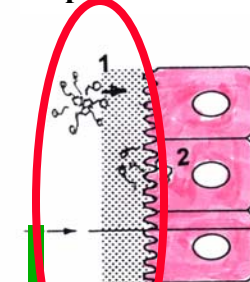
- *In vitro* assessment of bioaccessibility of soil-bound hydrocarbon residues in humans
 - LBNL's physiologically-based human small intestine (PHSI) extraction model: Assumptions and formulation
 - Predicting solubilization of soil-bound petroleum hydrocarbon residues in the human intestinal lumen: a working example
- *In vivo* investigation of fate of soil-bound hydrocarbon residues and metabolites in small mammals
 - LBNL's mass-balance mouse model
 - Kinetics of distribution and elimination of ^{14}C -hexadecane and ^{14}C -B[a]P and metabolites in mice: a working example
 - Kinetics of bioavailability of hydrocarbon residues in polluted PERF soils: a challenging example
- Summary

GI Uptake in humans of hydrocarbon residues

400 mg of soil
with petroleum
residues



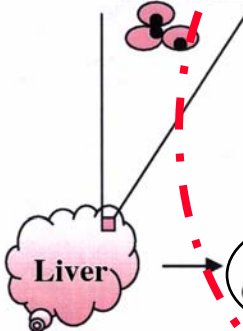
Petroleum
residues
crossing
epithelial cells



Fecal Excretion

Portal
Blood

CYP1A1 induction in
liver cells



Systemic
Circulation

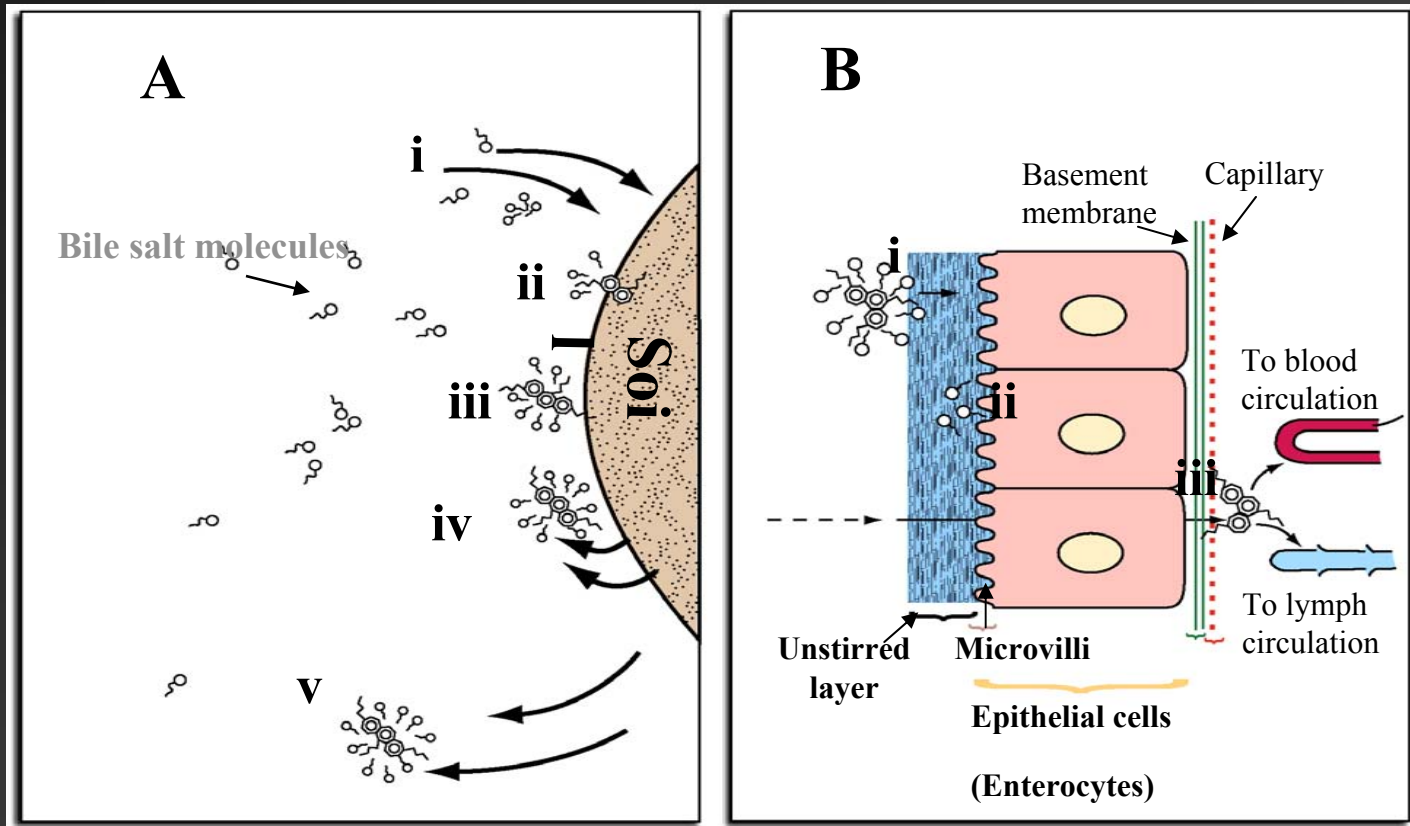
Metabolism
& Further
Elimination

Distribution

Urinary Excretion

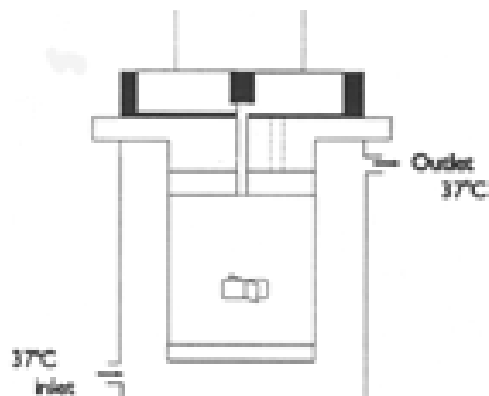
What are the Assumptions?

1. The gastrointestinal solubilization of soil-bound petroleum hydrocarbon residues in human gut is similar to the dissolution and uptake of hydrophobic drugs.
2. Interactions between petroleum hydrocarbons and endogenous digestive enzymes are negligible.



A Small Intestine Mimetic Device

1. Solubilization and release of soil-bound hydrocarbon residues in the upper small intestinal lumen



2. Hydrocarbon-containing micelles passing between microvilli to reach the surface area of the enterocytes



- ~ 0.4 g soil
- 250 mL synthetic upper small intestine (SUSI) digestive fluid
- Mixed for 4 hours at 20 rpm without headspace
- Filtered through a 0.45-micron filter

Holman et al., ES&T, 2002

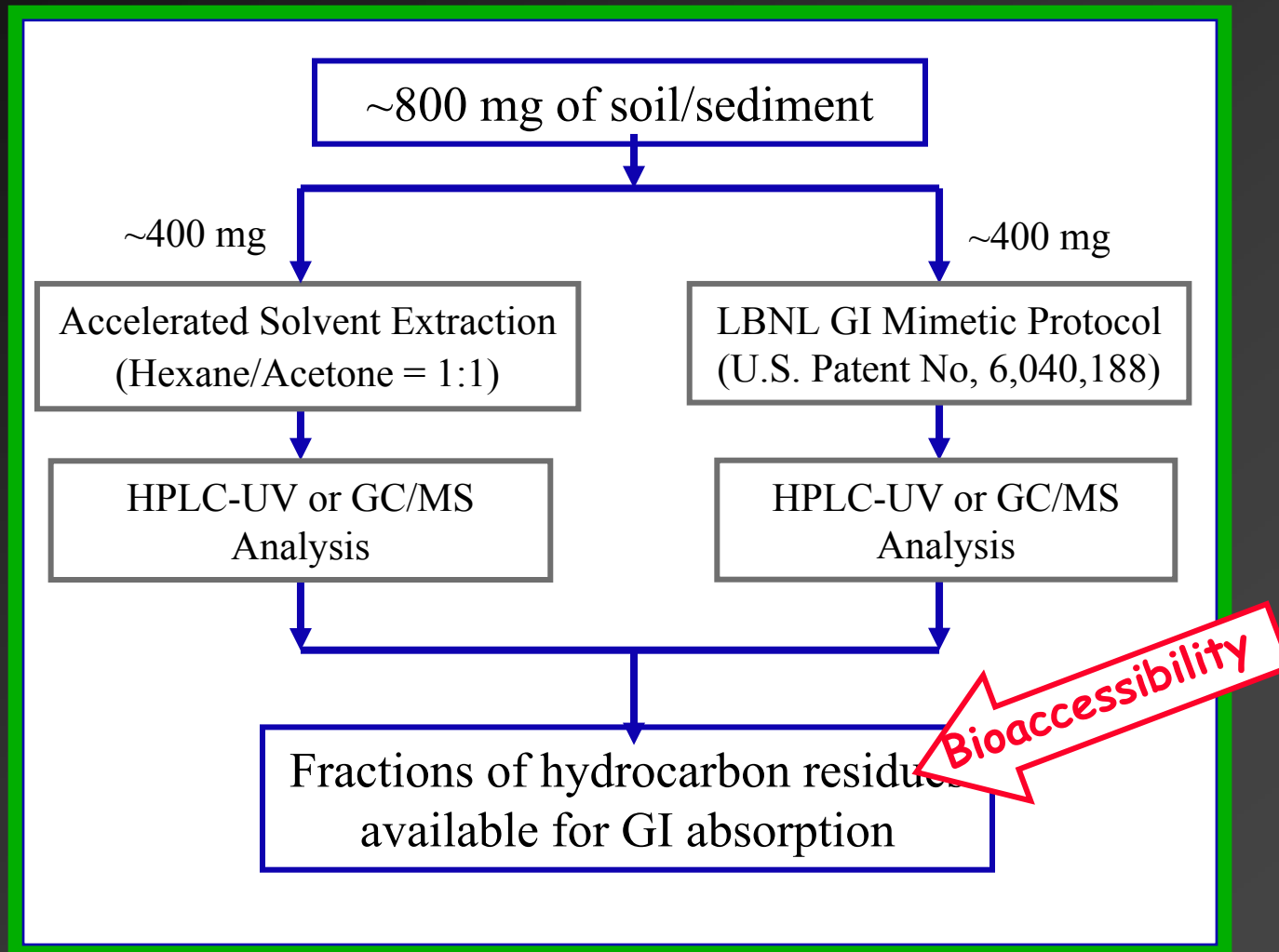
U.S. Patent No. 6,040,188

Synthetic Upper Small Intestine (SUSI) Digestive Fluid

Composition of fasted and fat digestion state digestive fluid systems		Concentration (mM)
Fasting (between meals/overnight fasting)	MBS Na ⁺ (from NaCl)	0.1 150.0
Fasting (gallbladder emptying phase)	MBS Na ⁺	5.0 150.0
Fat-digestion state	MBS MIL Na ⁺	20.0 (5.0 – 25.0) 30.0 150.0
Mixed Bile Salts (MBS)	Sodium glycocholate, Sodium glycochenodeoxycholate, Sodium glycodeoxycholate, Sodium glycolithocholate Disodium glycolithocholate sulfate Sodium taurocholate, Taurochenodeoxycholate, Sodium taurodeoxycholate, Sodium tauroolithocholate Disodium tauroolithocholate sulfate	100.0 23.5 23.5 16.0 0.7 3.0 12.0 12.0 8.0 0.3 1.0
Mixed Intestinal Lipids (MIL)	Cholesterol monohydrate Oleic acid Monoolein Diolein Lecithin	1.9 19.5 3.9 0.8 3.9

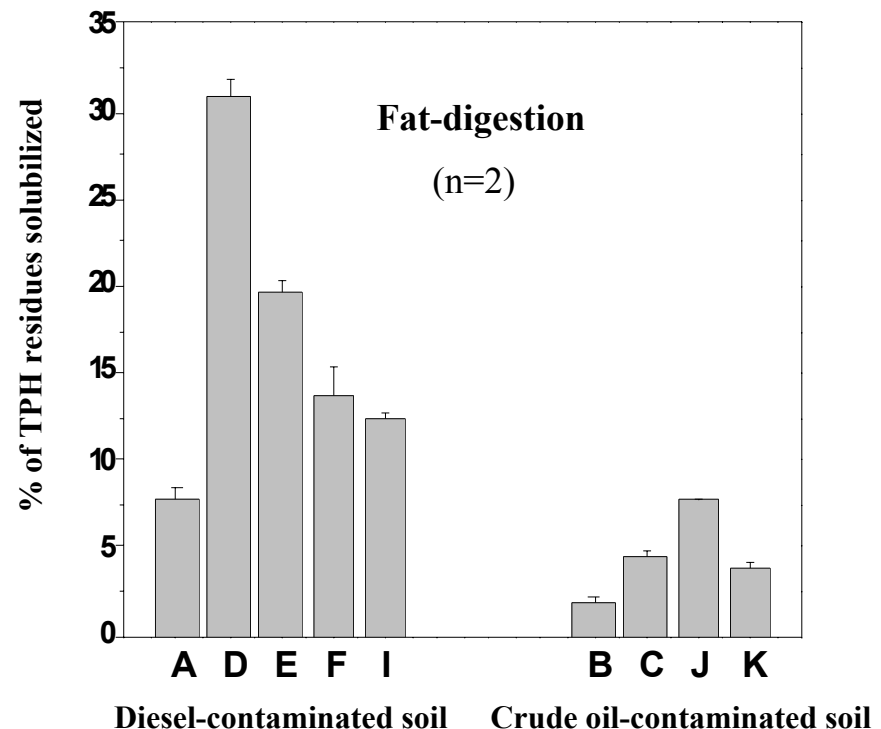
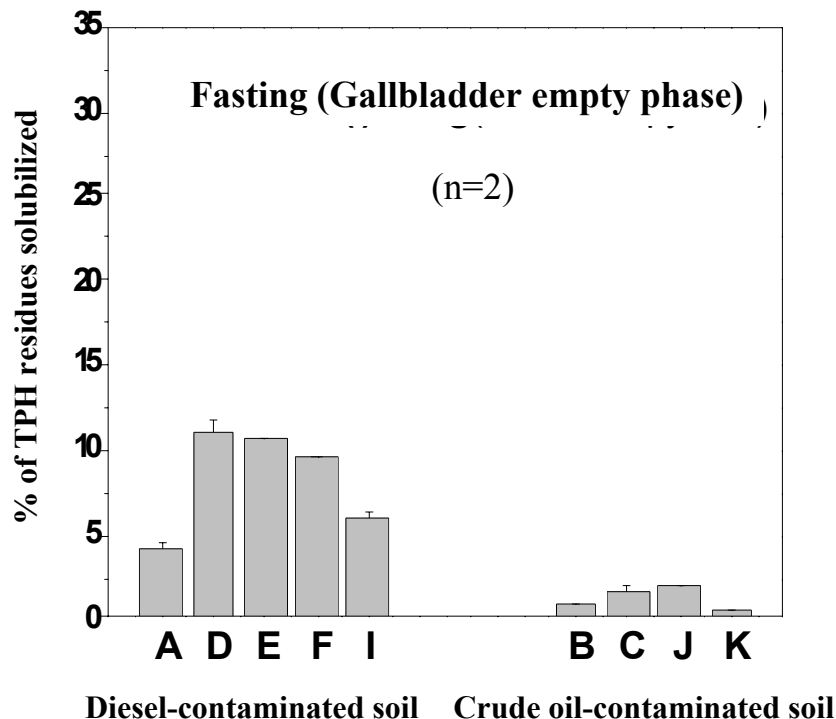
In Vitro Physiologically-Based Small Intestine (PSI) Extraction for Predicting Bioaccessibility of Hydrocarbon Residues

Procedure



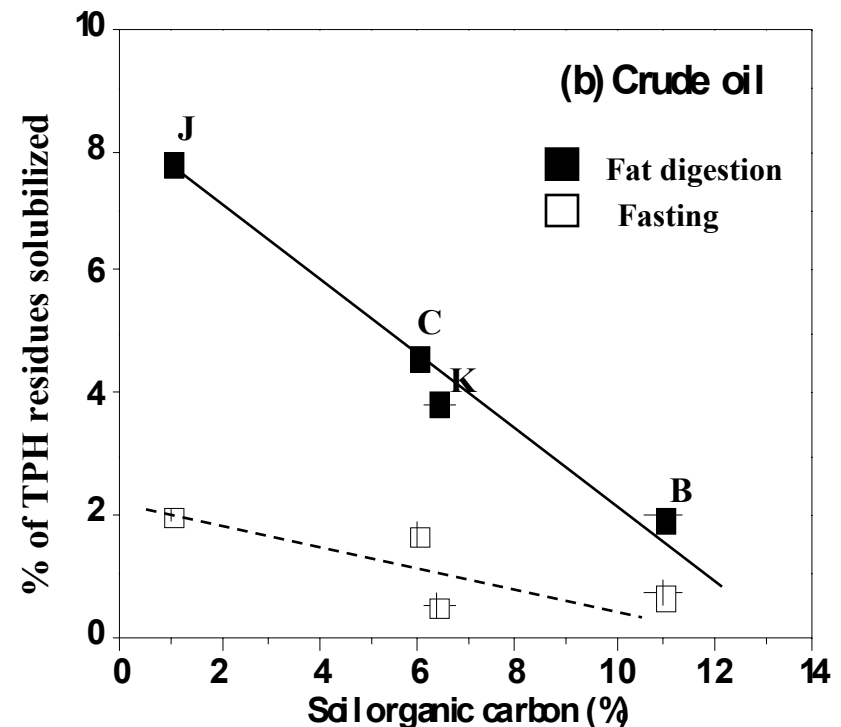
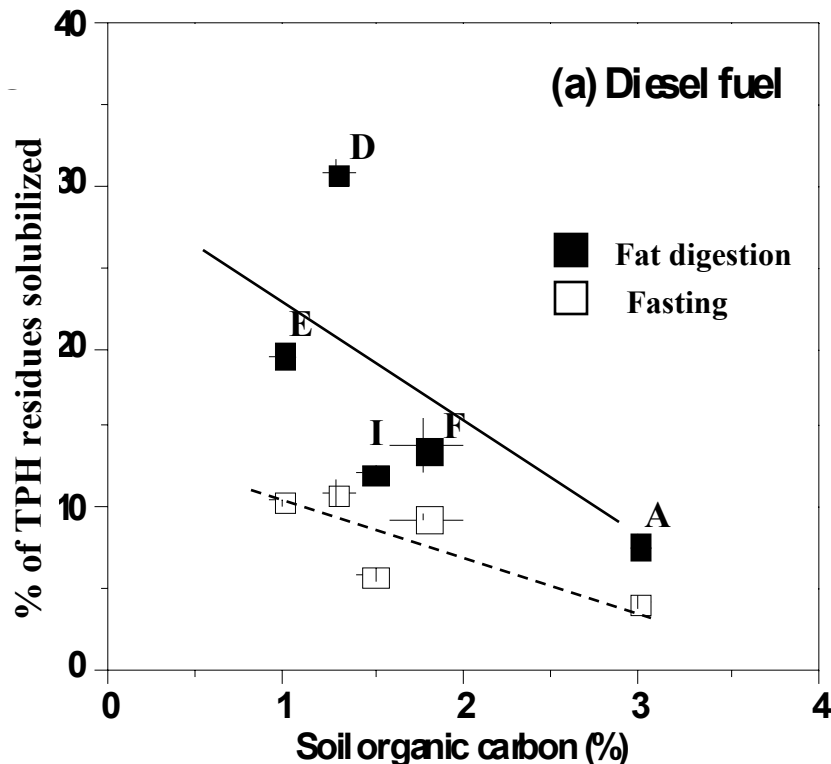
Application example: Predicting/comparing oral bioaccessibility of petroleum hydrocarbon residues from 9 sites selected by PERF

1. Comparing bioaccessibility of TPH residues in soils contaminated with diesel and crude oil during simulated (a) fasting state and (b) the fat-digestion state.



Application example: Predicting/comparing oral bioaccessibility of petroleum hydrocarbon residues from 9 sites selected by PERF

2. Evaluate bioaccessibility of TPH residues as a function of soil organic carbon during simulated fasting state and the fat-digestion state in soils contaminated with (a) diesel and (b) crude oil



LBNL's PHSI extraction model predicted that the *in vitro* human GI solubility of TPH residues:

- ⇒ increases for soil polluted with diesel than with crude oil,
- ⇒ increases for fat digestion state,
- ⇒ can be reduced by organic compounds.

LBNL's Mass-Balance *in vivo* Mouse Assay



An 86-day old female Swiss-Webster mouse (Simonsen Laboratory, Day Road, Gilroy, CA). She was weighed and lightly anesthetized (IsovetR, isofluorane, Schering-Plough Animal Health Corp., Union, NJ) before administration.

In vivo Mouse Assay

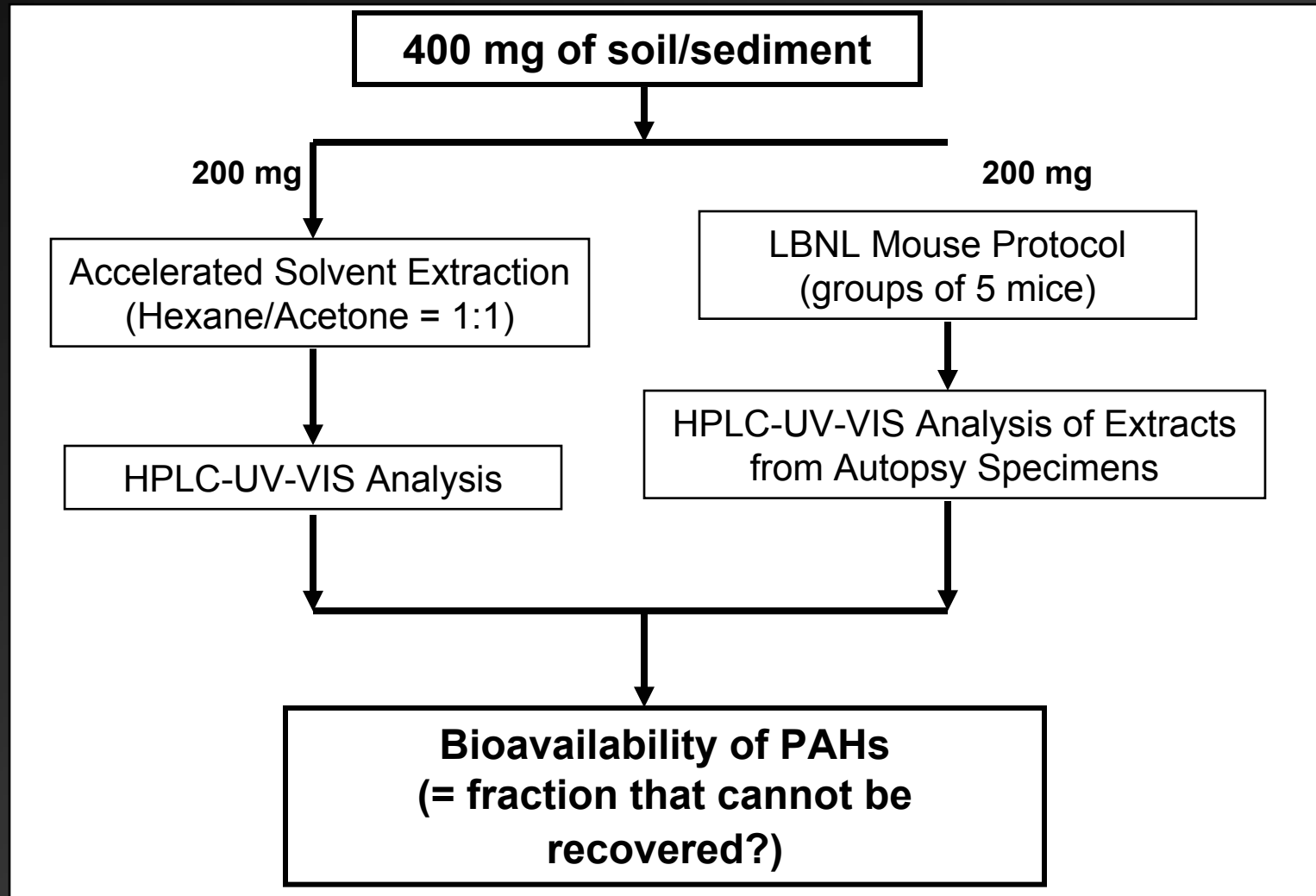
- Establish appropriated killing time by conducting transit-absorption experiments feeding mice ^{14}C -B(a)P and ^{14}C -hexadecane sorbed to model matrix and killing mice at $t = 15 \text{ min}, 30 \text{ min}, 1 \text{ hr}, 2 \text{ hr}, 4 \text{ hr}, 6 \text{ hr}, 8 \text{ hr}, 24 \text{ hr}$.
 - Oral administration (gavage) of a 0.2-mL suspension of ^{14}C -B(a)P- and ^{14}C -hexadecane sorbed PERF soil or an equal volume of the model vehicle control
 - Housing mice in a group of five in a stack cage or metabolism chamber
 - Killing mice at different time points and analyzed for the presence of ^{14}C -compounds and soil-bound TPHs in whole blood, plasma, kidneys, liver, full upper GI tract, full lower gut, carcass, fecal pellets, urine, and exhaled CO_2 .
- Use results from the transit-absorption experiments to design the mouse feeding experiment for environmental materials. ^{14}C -compounds are utilized as a procedural internal standard and introduced into the suspension shortly before oral administration.



The Optimal Experimental Setup



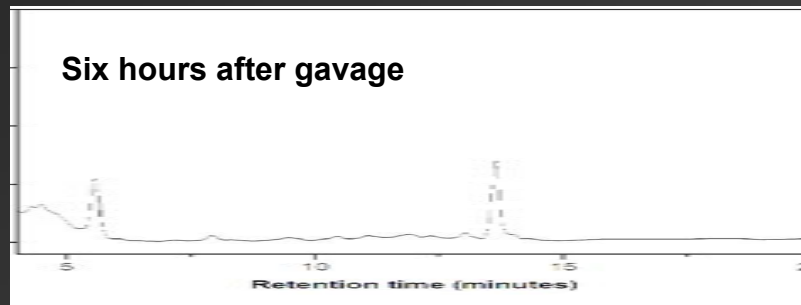
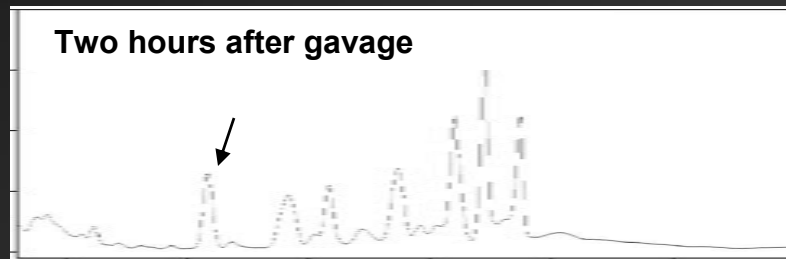
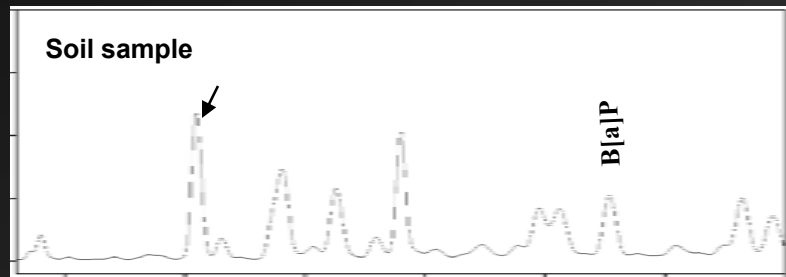
In vivo Procedure for Measuring Bioavailability



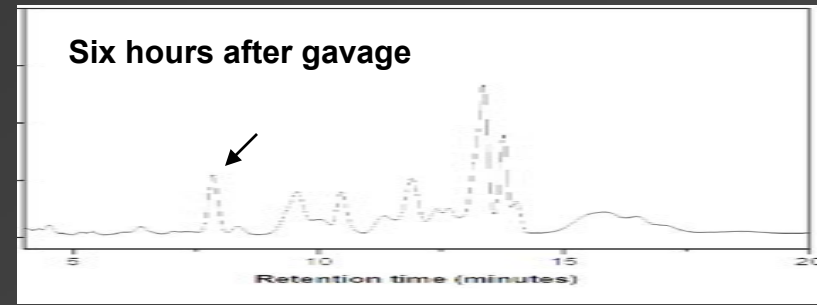
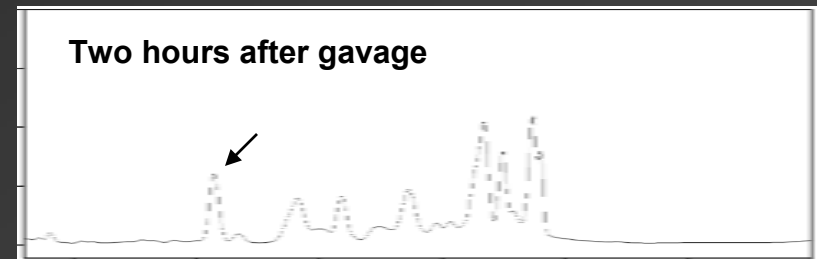
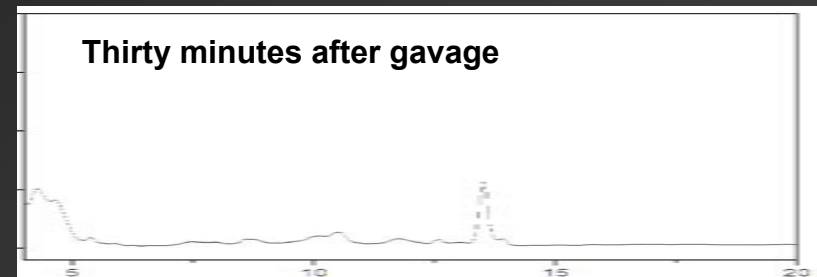
Example : Mouse Feeding Experiment Results- PAH Transformation in Mouse GI Gut Observed

Time evolving HPLC chromatograms of PAHs in mice showing PAH transformation inside the mouse gut.

Upper Gut



Lower Gut



Summary and Conclusions

- ⇒ **Benefits of a mass-balanced and physiologically-based *in vitro* GI protocol**
 - can be consistently applied to assess the potential exposure and risks of polluted soils and sediments from sites with a wide range of geochemical parameters
 - results are not complicated by metabolic transformation once inside the animal
 - cost saving and faster data availability
- ⇒ **Benefits of a mass-balanced *in vivo* mouse protocol and challenges**
 - provide a true picture of the exposure and potential risks of polluted soils and sediments
 - complement results from *in vitro* experiments
 - results are complicated by metabolic transformation
- ⇒ **Both assays yield similar qualitative results (e.g., matrix effects), but the effects are different and information can be utilized to complementing each other.**